Micropipettes
Adapted in part from Investigations in Molecular Cell Biology *lab manual, by Clare O'Connor, Boston College.*

A. Choosing the Micropipette
In many biology labs, especially microbiology and molecular biology, volumes are measured in the range of **microliters**. Remember these relationships between volume units:

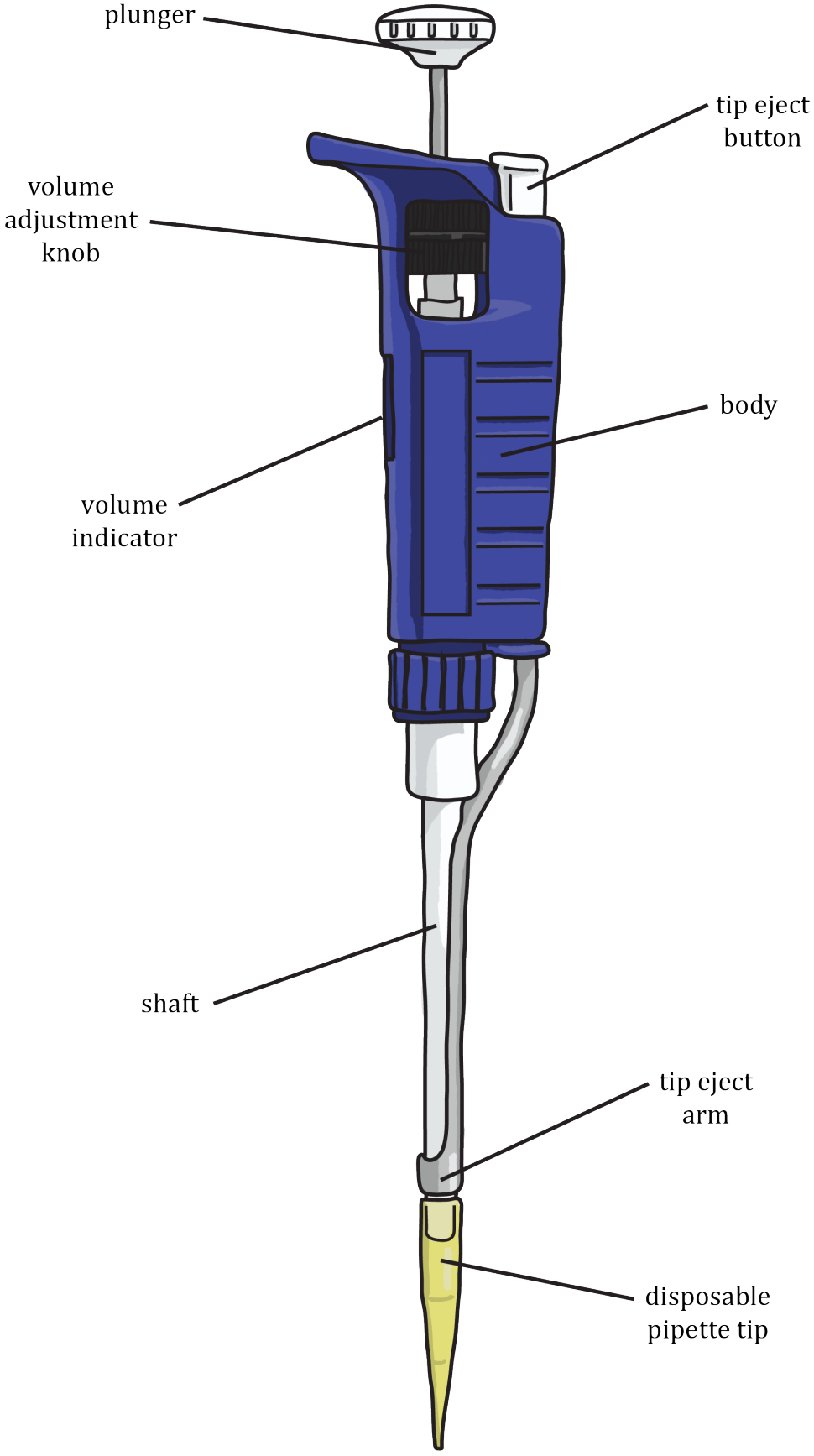
Unit	Symbol	Volume in Liters	Relationship
liter	L or l		
milliliter	mL or ml	0.001 L or $1x10^{-3}$ L	1000 ml in 1 L
microliter	µL, uL or ul	0.00000 L or $1x10^{-6}$ L	1000 ul in 1 mL

Micropipettes (also called Pipetmen or pipettors) are designed to precisely measure microliter-level volumes. There are three different types of micropipettes, each of which measures a different volume range. Each pipette is named after the largest volume that it is designed to transfer (in microliters):

Name	Maximum Volume	Range	Smallest Increment (µL)
P10	10 µL	1 µL – 10 µL	0.02
P20	20 µL	2 µL – 20 µL	0.02
P200	200 µL	20 µL – 200 µL	0.2
P1000	1000 µL	200 µL – 1000 µL	2.0

You may notice that there is some overlap in the ranges of the different micropipettes. In general, each micropipette is designed to be fairly accurate – however, this accuracy decreases when operating near the lowest values in their range. Because of this, you will have to choose the correct micropipette depending on the volume you wish to pipet. As a rule of thumb, always select the <u>smallest</u> volume micropipette that will transfer the volume you wish to pipet.

next page: Figure E1. A micropipette, with labeled parts. Although every model of micropipette is slightly different, each will have these major components. Based on the Gilson Pipetman®.



Appendix A (ii)

B. Setting the Micropipette

There are three numbers arranged vertically on the **volume indicator** (Figure E1). With each of the micropipettes, you will specify a volume to three digits by turning the **volume adjustment knob** (Figure E1). You will also be able to extrapolate between the lowest numbers with the hash marks on the lower dial.

Note: Never turn the volume adjustment knob beyond the upper or lower volume limits of the micropipette – this could damage the piston.

1. Based on the volume you intend to transfer, select the correct micropipette (See A. CHOOSING THE MICROPIPETTE).

2. Turn the volume adjustment knob to set the proper transfer volume. Twisting the knob clockwise will decrease the volume, and twisting the knob counter-clockwise will increase the volume.

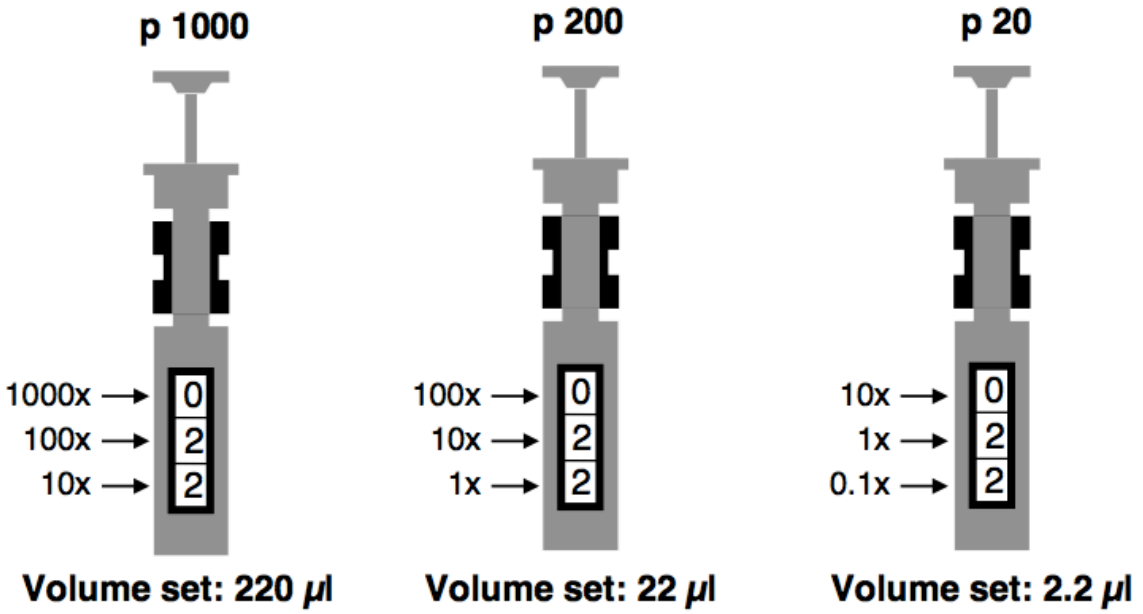


Figure E2. Transfer volumes are represented in different ways on different micropipettes. The same setting – "022" – represents a different volume on each micropipette. *Image source: Buffalo State Bio211 Micropipetting Lab*.

a. Each particular type of micropipette is set in a different way (Figure E2).

b. On a P1000 micropipette, the top number (red) on the volume indicator represents the number of thousands of microliters, the middle number (black) represents the number of hundreds of microliters, and the bottom number (black) represents the number of tens of microliters. Each tick mark at the bottom represents two microliters.

c. On a P200 micropipette, the top number (black) on the volume indicator represents the number of hundreds of microliters, the middle number (black) represents the number of tens of microliters, and the bottom number (black) represents the number of microliters. Each tick mark at the bottom represents two-tenths of a microliter.

d. On a P20 and P10 micropipette, the top number (black) on the volume indicator represents the number of tens of microliters, the middle number (black) represents the number of microliters, and the bottom number (red) represents the number of tenths of microliters. Each tick mark at the bottom represents two hundredths of a microliter.

C. Filling the Micropipette

1. Locate the box that contains the correct tips for the micropipette that you are using, and remove the lid. (Typically, the P1000 uses large blue or white tips, and both the P200 and P20 use small yellow tips. The P10 uses even smaller tips.) The lid will often be held on by a piece of white tape with black stripes – the black stripes indicate that the tips have been sterilized.

2. Place a tip on the micropipette by inserting the **shaft** (Figure E1) of the micropipette into the tip, and pressing down firmly. You are making an airtight seal between the tip and the shaft of the micropipette.

3. Replace the lid of the tip box to keep the remaining tips sterile. Avoid touching the tip, especially the thinner end, even with gloved hands, because the tips are sterile.

4. Depress the **plunger** (Figure E1) of the micropipette to the <u>first</u> stop (Figure E4).

Figure E3. Relative locations of first and second stops when depressing the plunger of a micropipette. *Image source: Boston College* Investigations in Molecular Cell Biology *lab manual*.

Note: The most common – and serious – operator error is depressing the plunger to the second stop before filling the micropipette tip. Do not do this.

5. Immerse the tip a few millimeters below the surface of the solution being drawn up into the pipette (Figure E4). Pipetting is most accurate when the pipette is held vertically. Always keep the angle of the pipet less than 20˚ from vertical.

6. Release the plunger slowly, drawing the solution up into the tip and allowing the tip to fill smoothly (Figure E4). Pause briefly to ensure that the full volume of sample has entered the tip. Do not let the plunger snap up. This is particularly important when transferring larger volumes, because a splash could contaminate the shaft of the micropipette. If you inadvertently contaminate the shaft, clean it immediately with a damp Kimwipe.

Note: Never rest a micropipette with fluid in its tip on the bench!

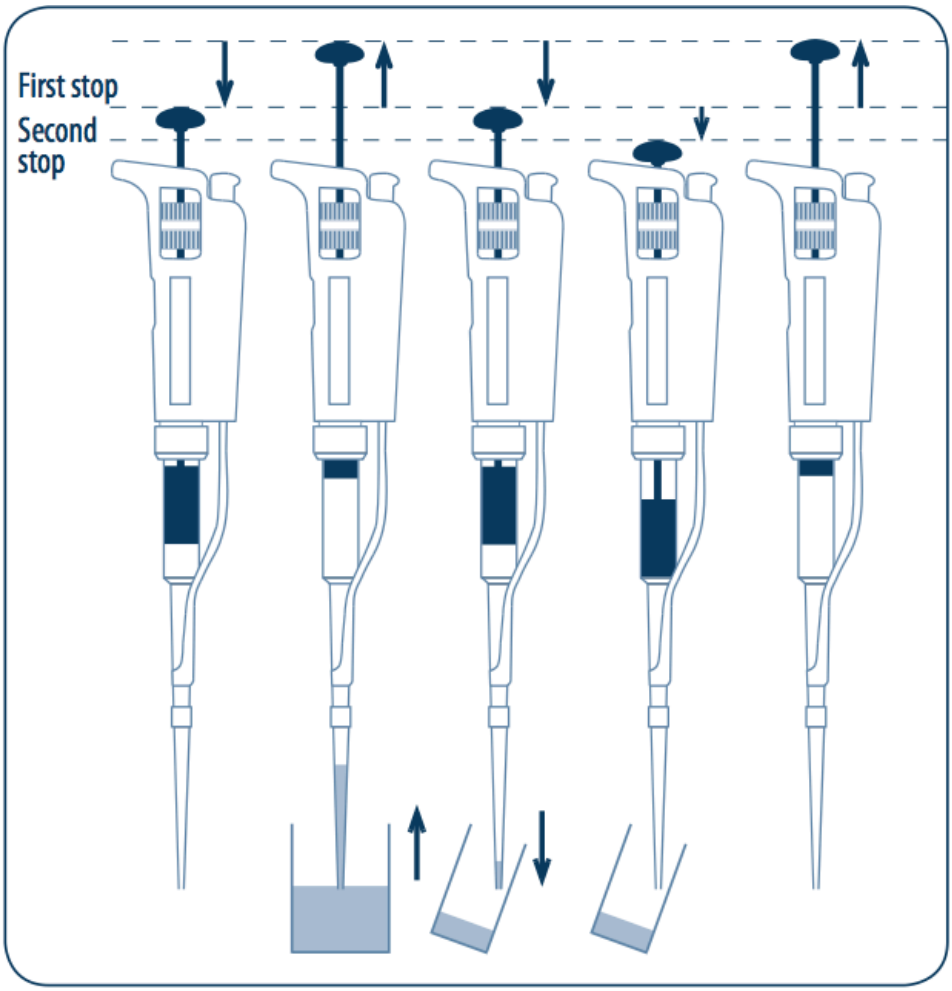


Figure E4. Steps in filling and dispensing a micropipette. Note that the first stop is used to fill the micropipette, and the second stop is only used to dispense. *Image source: Gilson Pipetman® User Guide*.

D. Dispensing the Contents of the Micropipette

1. Place the micropipette tip against the inside of the receiving container, or submerge the tip slightly into the liquid inside the receiving container (Figure E4). Surface tension will help to dispense the contents of the micropipette. Do not attempt to eject the contents of the micropipette into "thin air."

2. Depress the plunger smoothly to the first stop. Pause, then depress the plunger to the second stop (Figure E4). The contents of the pipette are mostly released at the first stop. The second stop ensures that you've released the "last drop."

3. Remove the micropipette from the receiving test tube, and press the **tip eject button** (Figure E1) to discard the tip into the trash.